DOCKET NO. 9511-057-27 DIV

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: Richard J. BUCALA, et al. ART UNIT: 1644

SERIAL NO.: 09/557,823 EXAMINER: Patrick J. Nolan

FILING DATE: April 25, 2000

FOR: METHOD FOR DETERMINING MIF CONTENT

DECLARATION OF ROBERT A. MITCHELL UNDER 37 C.F.R. §1.132

I, Robert A. Mitchell, do hereby declare the following:

1. I am one of the inventors of the subject matter of United States Patent Application Serial No. 09/557,823, filed on April 25, 2000, and am thus intimately familiar with the contents of the application, its prosecution before the United States Patent and Trademark Office, and the references cited therein. Attached is my curriculum vitae.

2. I have reviewed the specification and claims of the application. I have further reviewed the Office Actions issued on August 12, 2004, December 6, 2003, February 11, 2003, June 24, 2002 and December 4, 2001 in the above-captioned application and the reference of Ishizaka, et al. (US Patent No. 5,786,168, hereinafter "the '168 patent").

3. I have also reviewed the literature published since the filing of the original application relating to the macrophage migration inhibitory factor (MIF), and I have further reviewed all published literature relating to GIF.

4. I understand the Examiner's position to be that Ishizaka, et al. generally describe an antibody binds SEQ ID NO:38, which is identical to the amino acid sequence of SEQ ID NO:5 of the present invention, and one of skill in the art who practices the present invention using the

teaching of the specification would detect MIF and GIF simultaneously. However, the Examiner's position is incorrect for the reasons set forth below.

5. The present invention is directed to a diagnostic method for determining the amount of MIF in a sample using an anti-MIF antibody which binds specifically to the human MIF protein that has a molecular weight of approximately 12.5 kDa and has MIF biological activity.

6. In contrast, Ishizaka, et al. generally describe a method for producing a substantially pure biologically active antigen non-specific GIF. Ishizaka, et al. specifically disclose in the '168 patent that the recombinant GIF produced in *E. coli* failed to inhibit the migration of human monocytes, and "[t]he results indicated that rhGIF was different from MIF in biological activity." (Col. 46, line 47 to Col. 47, line 4).

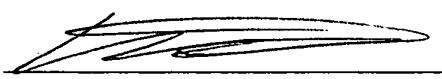
7. GIF is cited in the literature as having a molecular weight of 14 kDa, which is almost 2000 daltons larger than MIF (Tagaya et al. (1992) Biochemical characterization of murine glycosylation-inhibiting factor. *Proc. Natl. Acad. Sci. USA. 88*:9117-9121). The present MIF is a 12.5 kDa molecule. Further, Tagaya et al. show that GIF has a pI of 5.5, but MIF has two isoforms (Magi et al. (1998) Charge heterogeneity of macrophage migration inhibitory factor (MIF) in human liver and breast tissue. *Electrophoresis*, 19:2010-2013) and neither of these correspond to the pI for GIF. Also, MIF forms multimers giving rise to additional species of approximately 25 kDa (dimer) and 37 kDa (trimer). Neither of these additional species of a molecule is observed in the blots using an anti-GIF antibody.

8. Most important, in view of all published literature relating to the GIF, GIF is only described as an entity which is generated and purified from cells placed into tissue culture, hybridoma cells, or derived via recombinant means. There is no evidence, e.g., a western blot of serum or tissue extract probed with an anti-GIF antibody, that GIF exists in nature. In contrast, there are many publications showing the existence of MIF from *in vivo* tissue samples such as

serum or tissue extracts (e.g., pituitary). Since such a demonstration would have been easy to do, one can only come to the conclusion that it was never done or that the results were negative. Either way, there is no evidence that GIF naturally exists and, therefore, the question of detecting GIF in a diagnostic test is moot. A diagnostic for GIF has never been reduced to practice.

9. Also, in view of Ishizaka, et al. and numerous publications, it does not show the existence of a naturally-derived GIF. All GIF preparations mentioned in this patent and the publications are derived from cells (or hybridomas, which are modified cells that do not occur in nature) placed in an artificial environment (i.e., tissue culture) or are derived from a recombinant expression system. Furthermore, it is known to a person in the field that the only way to make GIF active is to modify or to mutate it. The GIF bioactivity assays commonly used by Ishizaka necessitates inactivating or mutating one or more free cysteines in the native molecule. In contrast, MIF is active as it is. GIF has therefore not been demonstrated to exist in nature (e.g., in a serum sample from a human) and may essentially exist only as an artifact of tissue culture. Based on this, it would be impossible to detect it in a diagnostic test as the molecule has not been proven to exist.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



Robert A. Mitchell

11/3/04

Date

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Education

1998	Albany Medical College, Ph.D, Experimental Pathology
1989	University of Iowa School of Medicine, BS, Immunology
1988	University of Iowa School of Medicine, Research Technology Program

Experience

2003-	*Assistant Professor,* Department of Biochemistry & Molecular Biology, University of Louisville
2003-	*Senior Faculty Member,* Graduate School, University of Louisville
2002-	*Assistant Professor,* Department of Medicine, University of Louisville
2002-	*Associate Member,* JG Brown Cancer Center, University of Louisville
1999-01	*Senior Scientist,* Picower Institute for Medical Research
1998-99	*Post-Doctoral Fellow,* Picower Institute for Medical Research
1994-98	*Graduate Student,* Albany Medical College
1992-94	*Senior Research Associate,* Picower Institute for Medical Research
1991-92	*Senior Research Assistant,* Rockefeller University
1989-91	*Principal Research Assistant,* University of Minnesota

Memberships

American Association for the Advancement of Sciences
Molecular Medicine Society

Committees/Service

MD/PhD Advisory Committee
Biochemistry & Molecular Biology Travel Award Committee
Biochemistry & Molecular Biology Exam 1 Committee
Keynote Speaker - Annual Kornhauser Library Friends Dinner
Keynote Speaker - Jefferson County Medical Society; Hyatt Regency Hotel
COBRE Grant Planning Committee
JG Brown Cancer Survivor's Day Participant
Core Cancer Cell Biology Curriculum Planning Committee
Translation Rounds Program - Member

NIH Study Section Reviewer

Ad hoc reviewer for:
Hemostasis & Thrombosis study section

Ad Hoc Reviewer

Clinical Cancer Research; *Journal of Histochemistry and Cytochemistry*; *Journal of Infectious Diseases; Journal of Pathology; Molecular Medicine*; *Molecular Cellular Biology; Oncology*

Grant Support

JG Brown Cancer Center Pilot Project Research Grant - PI
"Role of migration inhibitory factor (MIF) in *de novo* tumorigenesis" (Score – 165)
Status - Completed: 11/02 -11/03 - Direct costs: $30,000/year

NIH Centers of Biomedical Research Excellence Research Program Grant - Co-PI
"Development and testing of small molecule antagonists of MIF" (Score – 126)
Status - Active: 10/1/03 – 6/30/08 – Direct costs: $874,020

Kentucky Lung Cancer Research Program Grant - PI
"Promotion of NSCLC signaling and development by MIF" (Score – 189)
Status - Active: 7/1/03 - 6/30/06 – Direct costs: $291,612

NIH National Cancer Institute – PI
"Role of MIF in Rb inactivation and Tumorigenesis" (Score – 140)
Status – Active: 7/1/04 - 6/30/09 – Direct costs: $1,000,000

Mentoring

Graduate Students (5; 2 active); Medical Student (1; 1 active); Postdoctoral Fellows (3; 1 active); Research Assistants (4; 2 active); Hematology/Oncology Fellow (1); Internal Medicine Resident (1); High School Students (3)

Teaching

Molecular Basis of Cancer – "Molecular and Cellular Mechanisms of Angiogenesis"
Translational Rounds
Fellows Research Seminar Series

Patents

Combination Method for Treating Diseases Caused by Cytokine-Mediated Toxicity
(US # 6,030,615)
Diagnostic Assays for MIF
(US # 6,080,407)
Inducible 6-Phosphofructo-2-Kinase and the Warburg Effect
(US # 6,255,046)

Compounds Having MIF Antagonist Activity
(US # 6,492,248)
Composition Containing Anti-MIF Antibody
(US # 6,645, 493)
Methods and Agents for Immunization against Cancer
(Filed – Pending)

Publications

1) K.W. Harris, **R.A. Mitchell** and J.C. Winkelmann. (1991) Ligand Binding Properties of the Human Erythropoietin Receptor Extracellular Domain Expressed in E. Coli. *J. Biol. Chem.* (267) 15205-15209.

2) J. Bernhagen, T. Calandra, **R.A. Mitchell**, W. Voelter, A. Cerami and R. Bucala. (1993) Macrophage migration inhibitory factor (MIF) is a cytokine secreted by the pituitary after endotoxin stimulation. *Proceedings of Cytokines in Hematology, Oncology and Immunology,* Hanover, Germany June 1993.

3) J. Bernhagen, T. Calandra, **R.A. Mitchell**, S.B. Martin, K.J. Tracy, W. Voelter, K.R. Manogue, A. Cerami and R. Bucala. (1993) Macrophage migration inhibition factor (MIF) is a pituitary-derived cytokine that potentiates lethal endotoxaemia. *Nature* (365) 756-759.

4) T. Calandra, J. Bernhagen, **R.A. Mitchell**, and R. Bucala. (1994) The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor. *J. Exp. Med.* (79)6 1895-1902.

5) J. Bernhagen, **R.A. Mitchell**, T. Calandra, W. Voelter, A Cerami, and R. Bucala. (1994) Purification, bioactivity, and secondary structure analysis of mouse and human macrophage migration inhibitory factor (MIF). *Biochemistry* (33)47 14144-14155.

6) **R.A. Mitchell**, M. Bacher, J. Bernhagen, T. Pushkarskaya, M.F. Seldin and R. Bucala. (1995) Cloning and characterization of the gene for mouse macrophage migration inhibitory factor. *J. Immunol.* (154)8 3863-3870.

7) R. Bucala, **R.A. Mitchell**, K. Arnold, T. Innerarity, H. Vlassara and A. Cerami. (1995) Identification of the major site of apolipoprotein B modification by advanced glycosylation end products blocking uptake by the low density lipoprotein receptor. *J. Biol. Chem.* (270)18 10828-10832.

8) B. Sherry, G. Zybarth, M. Alfano L. Dubrovsky, **R.A. Mitchell**, D. Rich, P. Ulrich, R. Bucala, A. Cerami and M. Bukrinsky. (1998) Role of cyclophilin A in the uptake of HIV-1 by macrophages and T lymphocytes. *Proc. Natl. Acad. Sci.* 95(4) 1758-1763.

9) J. Chesney, **R.A. Mitchell**, F. Benigni, M. Bacher, L. Spiegel, Y. Al-Abed, J.H. Han, C. Metz and R. Bucala. (1999) An inducible gene product for 6-phosphofructo-2-kinase with an AU-rich instability element: Role in tumor cell glycolysis and the Warburg effect. *Proc. Natl. Acad. Sci.* 96(6) 3047-3052.

10) **R.A. Mitchell**, C. Metz, T. Peng and R. Bucala. (1999) Sustained MAP kinase and $cPLA_2$ activation by macrophage inhibitory factor (MIF): Regulatory role in cell proliferation and glucocorticoid action. *J. Biol. Chem.* 274 18100-18106.

11) **R.A. Mitchell** and R. Bucala. (2000) Tumor growth-promoting properties of macrophage migration inhibitory factor (MIF). *Semin. Cancer Biol.* 10(5):359-66.

12) A.V. Sampey, P.H. Hall, **R.A. Mitchell**, C.N. Metz and E.F. Morand. (2001) Regulation of synoviocyte phospholipase A2 and cyclooxygenase 2 by macrophage migration inhibitory factor. *Arthritis Rheum.* 44(6) 1273-80.

13) P.D. Senter, Y. Al-Abed, C.N. Metz, F. Benigni, **R.A. Mitchell**, C.G. Gartner, S.D. Nelson, G.J. Todaro and R. Bucala. (2002) Inhibition of macrophage migration inhibitory factor (MIF) tautomerase and biological activities by acetaminophen metabolites. *Proc. Natl. Acad. Sci.* 99(1):144-149.

14) **R.A. Mitchell***, H. Liao, J. Chesney, G. Fingerle-Rowson, J. Baugh, J. David, R. Bucala. (2002) MIF sustains macrophage pro-inflammatory function by inhibiting p53: Regulatory role in the innate immune response. *Proc. Natl. Acad. Sci.* 99(1):345-350. *Corresponding author

15) A. Dios, **R.A. Mitchell**, B. Aljabari, J. Lubetsky, K.A. O'Connor, H. Liao, P.D. Senter, K.R. Manogue, E. Lolis, C.N. Metz, R. Bucala, D.J. Callaway and Y. Al-Abed. (2002) Inhibition of MIF bioactivity by rational design of pharmacological inhibitors of MIF tautomerase activity. *J. Med. Chem.* 45(12):2410-2416

16) J.B. Lubetsky, A. Dios, J. Han, B. Aljabari, B. Ruzsicska, **R.A. Mitchell,** E. Lolis and Y. Al-abed. (2002) The tautomerase activity of MIF is a potential target for discovery of novel anti-inflammatory agents. *J. Biol. Chem.* 277(28):24976-82.

17) T. Atsumi, J. Chesney, C. Metz, L. Leng, S. Donnelly Z. Makita, **R.A. Mitchell**, and R. Bucala. (2002) High Expression of Inducible 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase (iPFK-2; PFKFB3) in Human Cancers. *Cancer Res.* 62(20):5881-7.

18) H. Liao, R. Bucala and **R.A. Mitchell**. (2003) Adhesion-dependent signaling by Macrophage Migration Inhibitory Factor (MIF). *J. Biol. Chem.* 278(1):76-81

19) D. Lacey, A. Sampey, **R.A. Mitchell**, L. Santos, M. Leech and E. Morand. (2003) Control of fibroblast-like synoviocyte proliferation by macrophage migration inhibitory factor. *Arthritis Rheum.* 48(1):103-9.

20) O. Petrenko, G. Fingerle-Rowson, T. Peng, **R.A. Mitchell** and C.N. Metz. (2003) MIF-deficiency is associated with altered cell growth and reduced susceptibility to Ras mediated transformation. *J. Biol. Chem.* 278(13):11078-11085

21) B. Rendon-Mitchell, M. Ochani, J. Li, J. Han, H. Wang, S. Susarla, C. Czura, **R.A. Mitchell**, A. Sama, K. Tracey and H. Wang. (2003) IFNγ induces HMGB1

release partially through a TNF-dependent mechanism. *J. Immunol.* 170(7)3890-3897.

22) L. Leng, C. Metz, Y. Fang, J. Xu, S. Donnelly, J. Baugh, T. Delohery, Y. Chen, **R.A. Mitchell** and R. Bucala. (2003) MIF signal transduction initiated by binding to CD74. *J. Exp. Med.* 197(11):1467-1476.

23) G. Fingerle-Rowson, O. Petrenko, C.N. Metz, T.G Forsthuber, **R.A. Mitchell**, R. Huss, U. Moll, W. Muller and R. Bucala. (2003) The p53-dependent effects of macrophage migration inhibitory factor revealed by gene targeting. *Proc Natl Acad Sci U S A.* 100(16):9354-9359.

24) **R.A. Mitchell**, B.G. Brewer and J.W. Eaton. (2004) Embryonic Vaccines Against Cancer: An Early History. *Cancer* (Submitted)

25) J.D. Swant, B.E. Rendon, M. Symons and **R.A. Mitchell**. (2004) RhoA GTPase-dependent signaling is required for MIF-mediated expression of cyclin D1. (Submitted)

Invited Reviews

1) **R.A. Mitchell** and R. Bucala. (2000) Tumor growth-promoting properties of macrophage migration inhibitory factor (MIF). *Semin. Cancer Biol.* 10(5):359-66.

2) **R.A. Mitchell.** (2004). Mechanisms and effectors of MIF-dependent promotion of tumorigenesis. *Cell. Signal.* 16(1):13-19.